

In the Specification:

Please amend the specification without prejudice as follows:

Please delete the paragraph beginning at line 3 of page 1 and ending at line 5 of page 1 and insert the following paragraph in place thereof:

-- CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a Division of U.S. Patent Application No. 09/831,630, filed May 10, 2001, which is a U.S. National Stage Application under 35 U.S.C. § 371 of International Application No. PCT/US99/26807, filed November 11, 1999, which claims priority in the United States under 35 U.S.C. § 119 to Denmark Application No. PA 1998 01483 filed November 13, 1998, which application is incorporated by reference herein in its entirety. --

Please delete the paragraph beginning at line 21 of page 1 and ending at line 25 of page 3 and insert the following paragraph in place thereof:

-- A family of UDP-galactose;  $\beta$ -N-acetyl-glucosamine  $\beta$ 1-3galactosyltransferases ( $\beta$ 3Gal-T's) was recently identified (Amado, M., Almeida, R., Carneiro, F., et al. A family of human  $\beta$ 3-galactosyltransferases: characterisation of four members of a UDP-galactose  $\beta$ -N-acetylglucosamine/ $\beta$ -N-acetylgalactosamine  $\beta$ 1,3-Galactosyltransferase family. *J. Biol. Chem.* 273:12770-12778, 1998; Kolbinger, F., Streiff, M.B. and Katopodis, A.G. Cloning of a human UDP-galactose:2-acetamido-2-deoxy-D-glucose  $\beta$ 3-galactosyltransferase catalysing the formation of type 1 chains. *J. Biol. Chem.* 273:433-440, 1998; Hennett, T., Dinter, A., Kuhnert, P., Mattu, T.S., Rudd, P.M. and Berger, E.G. Genomic cloning and expression of three murine UDP-galactose:  $\beta$ -N-acetylglucosamine  $\beta$ 1,3-galactosyltransferase genes. *J. Biol. Chem.* 273:58-65, 1998; Miyaki, H., Fukumoto, S., Okada, M., Hasegawa, T. and Furukawa, K. Expression

cloning of rat cDNA encoding UDP-galactose 4-epimerase that determines the expression of G(D1b)/G(M1)G(A1). *J. Biol. Chem.* 272:24794-24799, 1997). Three genes within this family,  $\beta$ 3Gal-T1, -T2, and -T3, encode  $\beta$ 3galactosyltransferases that form the Gal $\beta$ 1-3GlcNAc linkage. The type 1 chain Gal $\beta$ 1-3GlcNAc sequence is found in both N- and O-linked oligosaccharides of glycoproteins and in lactoseries glycosphingolipids, where it is the counterpart of type 2 Gal $\beta$ 1-4GlcNAc poly-*N*-acetyllactosamine structures (Kobata. A. Structures and functions of the sugar chains of glycoproteins. *Eur J Biochem* 209:483-501, 1992.). Type 1 chain structures are found mainly in endodermally derived epithelia, whereas the type 2 chains are found in ecto- and mesodermally derived cells including erythrocytes (Oriol, R., Le Pendu, J. and Mollicone, R. Genetics of ABO, H, Lewis, X and related antigens. *Vox Sanguinis* 51:161-171, 1986; Clausen, H. and Hakomori, S. ABH and related histo-blood group antigens; immunochemical differences in carrier isotypes and their distribution. *Vox Sanguinis* 56:1-20, 1989). Normal gastro-intestinal epithelia express mainly type 1 chain glycoconjugates, while type 2 chain structures are predominantly expressed in tumors (Hakomori, S. Aberrant glycosylation in tumors and tumor-associated carbohydrate antigens. Tumor malignancy defined by aberrant glycosylation and sphingo(glyco)lipid metabolism. *Advances in Cancer Research* 52:257-331, 1989; Hakomori, S. Tumor malignancy defined by aberrant glycosylation and sphingo(glyco)lipid metabolism. *Cancer Res* 56:5309-5318, 1996). It is of considerable interest to define the gene(s) responsible for formation of these core structures in normal and malignant epithelia. Several characteristics of the three previously described  $\beta$ 3Gal-Ts capable of forming type 1 chain structures suggest that these are not the major enzyme(s) involved in type 1 chains synthesis in epithelia: (i) Northern analysis indicates that  $\beta$ 3Gal-T1 and -T2 are exclusively expressed in brain (Amado, M., Almeida, R., Carneiro, F., et al. A family of human  $\beta$ 3-galactosyltransferases: characterisation of four members of a UDP-galactose  $\beta$ -N-acetylglucosamine/ $\beta$ -N-acetylgalactosamine  $\beta$ 1,3-Galactosyltransferase family. *J. Biol. Chem.* 273:12770-12778, 1998; Kolbinger, F., Streiff, M.B. and Ktopodis, A.G. Cloning of a human UDP-galactose:2-acetamido-2-deoxy-D-glucose  $\beta$ 3-galactosyltransferase

catalysing the formation of type 1 chains. *J. Biol. Chem.* 273:433-440, 1998; Hennett, T., Dinter, A., Kubnert, P., Mattu, T.S., Rudd, P.M. and Berger, E.G. Genomic cloning and expression of three murine UDP-galactose:  $\beta$ -N-acetylglucosamine/ $\beta$ 1,3-galactosyltransferase genes. *J. Biol. Chem.* 273:58-65, 1998); (ii) although  $\beta$ 3Gal-T3 has a wider expression pattern it is not detected in several tissues including colon and it is weakly expressed in gastric mucosa (Amado, M., Almeida, R., Carneiro, F., et al. A family of human  $\beta$ 3-galactosyltransferases: characterisation of four members of a UDP-galactose  $\beta$ -N-acetylglucosamine/ $\beta$ -N-acetylgalactosamine  $\beta$ 1,3-Galactosyltransferase family. *J. Biol. Chem.* 273:12770-12778, 1998; Kolbinger, F., Streiff, M.B. and Ktopodis, AG. Cloning of a human T.JDP-galactose:2- acetamido-2-deoxy-D-glucose  $\beta$ 3-galactosyltransferase catalysing the formation of type 1 chains. *J. Biol. Chem.* 273:433-440, 1998); (iii) the kinetic properties of recombinant enzymes are not consistent with those reported for  $\beta$ Gal-T activities in epithelia (Sheares, B.T., Lau, J.T. and Carlson, D.M. Biosynthesis of galactosyl-beta 1,3-N- acetylglucosamine. *J. Biol. Chem.* 257:599-602, 1982; Holmes, E.H. Characterization and membrane organization of ~~beta 1-3 and beta 1-4~~ galactosyltransferases beta 1-3 and beta 1-4 galactosyltransferases from human colonic adenocarcinoma cell lines Cob 205 and SW403: basis for preferential synthesis of type 1 chain lacto-series carbohydrate structures. *Arch Biochem Biophys* 270:630-646, 1989); and (iv) the acceptor substrate specificities of  $\beta$ 3Gal-T1, -T2, or -T3 do not include the mucin-type core 3 structure (Amado, M., Almeida, R., Carneiro, F., et al. A family of human  $\beta$ 3-galactosyltransferases: characterisation of four members of a UDP-galactose  $\beta$ -N-acetylglucosamine/ $\beta$ -N-acetylgalactosamine  $\beta$ 1,3-Galactosyltransferase family. *J. Biol. Chem.* 273:12770-12778, 1998; Hennett, T., Dinter, A., Kuhnert, P., Mattu, T.S., Rudd, P.M. and Berger, E.G. Genomic cloning and expression of three murine UDP-galactose:  $\beta$ 3-N-acetylglucosamine  $\beta$ 1,3-galactosyltransferase genes. *J. Biol. Chem.* 273:58-65, 1998), which was previously found to be a highly efficient substrate for  $\beta$ 3Gal-T activity isolated from porcine trachea (Sheares, B.T. and Carison, D.M. Characterization of UDP-galactose:2-acetamido-2-deoxy-D- glucose 3 beta-galactosyltransferase from pig trachea. *J. Biol. Chem.* 258:9893-9898, 1983). --

Please delete the paragraph beginning at line 29 of page 19 and ending at line 3 of page 21 and insert the following paragraph in place thereof:

-- These relatively high  $K_m$ s for donor substrates are significantly different from those reported for  $\beta$ Gal-T1 and -T2 (90 and 37  $\mu$ M, respectively) (Amado, M., Almeida, R., Carneiro, F., et al. A family of human  $\beta$ 3-galactosyltransferases: characterisation of four members of a UDP-galactose  $\beta$ -N-acetylglucosamine/ $\beta$ -N-acetylgalactosamine  $\beta$ 1,3-Galactosyltransferase family. *J. Biol. Chem.* 273:12770-12778, 1998). Interestingly, activity of the full length coding construct of  $\beta$ 3Gal-T5 analyzed in Triton CF-54 homogenates of infected insect cells showed a lower apparent  $K_m$  of 33  $\mu$ M for the donor substrate (not shown). The purified  $\beta$ 3Gal-transferase activity analyzed by Sheares, *et al.* (Shears, B.T. and Carison, D.M. Characterization of UDP-galactose:2-acetamido-2-deoxy-D- glucose 3 beta-galactosyltransferase from pig trachea. *J. Biol. Chem.* 258:9893-9898, 1983) is, however, likely to represent a truncated proteolytically cleaved form that is often found with affinity-purified glycosyltransferase preparations (Clausen, H., White, T., Takio, K., et al. Isolation to homogeneity and partial characterization of a histo-blood group A defined Fuc ~~alpha 1—2Gal alpha 1—~~ 3-N-acetylgalactosaminyltransferase alpha 1-2Gal alpha 1-3-N-acetylgalactosaminyltransferase from human lung tissue. *J. Biol. Chem.* 265:1139-1145, 1990). Moreover, Holmes (Holmes, E.H. Characterization and membrane organization of ~~beta 1—3- and beta 1—4- galactosyltransferases~~ beta 1-3- and beta 1-4- galactosyltransferases from human colonic adenocarcinoma cell lines Cob 205 and SW403: basis for preferential synthesis of type 1 chain lacto-series carbohydrate structures. *Arch Biochem Biophys* 270:630-646, 1989) reported that non-purified  $\beta$ 3Gal-T activity from Colo205 cells had an apparent  $K_m$  for UDP-Gal of 48  $\mu$ M using glycolipids as acceptor substrate. This preparation may contain both full and secreted forms of transferases. The recombinant full length form of  $\beta$ 3Gal-T5 resembled the recombinant secreted form in all other aspects tested. The porcine  $\beta$ 3Gal-transferase activity has an apparent  $K_m$  for core 3 of 2.4 mM and  $\beta$ 3Gal-T5 exhibited an apparent

Km for core 3 of 2.8 mM. Holmes (Holmes, E.H. Characterization and membrane organization of ~~beta 1—3 and beta 1—4 galactosyltransferases~~ beta 1-3- and beta 1-4-galactosyltransferases from human colonic adenocarcinoma cell lines Cob 205 and SW403: basis for preferential synthesis of type 1 chain lacto-series carbohydrate structures. *Arch Biochem Biophys* 270:630-646, 1989) reported a Km for Lc<sub>3</sub>Cer of 13μM for β3Gal-T activity from Colo205 cells. The best substrate identified for β3Gal-T5 was β-D-GlcNAc(1-3)-D-β-Gal-1-Me [apparent Km of 1.8 mM (Table V)]. This is similar to the apparent Km of 2.9 mM for human colonic β3 Gal-T activity for β-D-GlcNAc(1-3)-D-β-Gal(1-4)-D-β-Glc (Seko, A., Ohkura, T., Kitamura, H., Yonezawa, S., Sato, E. and Yamashita, K. Quantitative differences in GlcNAc:beta1-->3 and GlcNAc:beta1-->4 galactosyltransferase activities between human colonic adenocarcinomas and normal colonic mucosa. *Cancer Res* 56:3468-3473, 1996). β3Gal-T5 showed strict donor substrate specificity for UDP-Gal and did not utilize UDP-GalNAc or UDPGlcNAc with the acceptor substrates tested (data not shown). --

Please delete the paragraph beginning at line 15 of page 28 and ending at line 20 of page 28 and insert the following paragraph in place thereof:

-- Nucleic acids encoding wild-type or variant polypeptides may also be introduced into cells by recombination events. For example, such a sequence can be introduced into a cell, and thereby effect homologous recombination at the site of an endogenous gene or a sequence with substantial identity to the gene. Other ~~recombination-based~~ recombination-based methods such as nonhomologous recombinations or deletion of endogenous genes by homologous recombination may also be used. --

Please delete the paragraph beginning at line 6 of page 37 and ending at line 5 of page 38 and insert the following paragraph in place thereof:

-- The kinetic properties were determined with partially purified, secreted forms of the enzymes. Semipurification of enzymes from serum-free medium of infected

High-Five™ cells was performed by sequential Amberlite, DEAE-Sephacel and 5-Sepharose chromatography as described previously (Wandall, H.H., Hassan, H., Mirgorodskaya, E., et al. Substrate specificities of three members of the human UDP-N-acetyl-alpha-D-galactosamine:Polypeptide Nacetylglactosaminyltransferase family, GalNAc-T1, -T2, and -T3. *J. Biol. Chem.* 272:23503-23514, 1997). Comparisons of enzymes were performed relatively to the activity obtained with  $\beta$ GlcNAc-Bzl (Tables II and III). Full length enzymes were assayed with 1% Triton CF54 homogenates of washed cells. Enzyme assays were performed in 50  $\mu$ l total reaction mixtures containing 25 mM Cacodylate (pH 7.5), 10 mM  $MnCl_2$ , 0.25% Triton X-100, 100  $\mu$ M UDP-[ $^{14}C$ ]-Gal (2,600 cpm/nmol) (Amersham), and varying concentrations of acceptor substrates (Sigma) (see Table I for structures). Reaction products were quantified by Dowex-1 chromatography. Assays with glycoproteins were performed with the standard reaction mixture modified to contain 150  $\mu$ M UDP-Gal, 54 mM NaCl, and 0.5 mg ovalbumin, asialo-agalacto-fetuin, orosomucoid, or bovine submaxillary mucin acceptor substrates obtained as previously described (Schwientek, T., Almeida, R., Levery, S.B., Holmes, E., Bennett, E.P. and Clausen, H. Cloning of a novel member of the UDP-galactose:  $\beta$ -N-acetylglucosamine  $\beta$ 1,4- galactosyltransferase family,  $\beta$ 4Gal-T4, involved in glycosphingolipid biosynthesis. *J. Biol Chem.* 273:29295-29305, 1998). The transfer of Gal was evaluated after acid precipitation by filtration through Whatman GF/C glass fibre filters. Assays to determine  $K_m$  of acceptor substrates and donor substrates were modified to include 200  $\mu$ M UDP-[ $^{14}C$ ]-Gal (2,600 cpm/nmol) or 30mM GlcNAc $\beta$ -benzyl. Assays with glycolipid acceptors were conducted as previously described (Holmes, E.H. Characterization and membrane organization of ~~beta 1—3— and beta 1—4—galactosyltransferases~~ beta 1-3- and beta 1-4- galactosyltransferases from human colonic adenocarcinoma cell lines Cob 205 and SW403: basis for preferential synthesis of type 1 chain lacto-series carbohydrate structures. *Arch Biochem Biophys* 270:630-646, 1989) in reaction mixtures containing 2.5  $\mu$ mol HEPES buffer, pH 7.2,  $\mu$ mol  $MnCl_2$ , 100  $\mu$ g taurodeoxycholate or Triton CF-54, 20  $\mu$ g acceptor glycolipid, 15 nmol UDP-[ $^{14}C$ ]-galactose (13,000 cpm/nmol) and enzyme in a total volume of 100  $\mu$ l. Conditions for incubation and product isolation were as previously described (Id.). –